

**A Note concerning the Collar Cavities of the  
Larval Amphioxus.**

By

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With Plate 18.

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INTRODUCTION.

Notwithstanding the great volume of literature dealing with the development of *Amphioxus*, there are many points still outstanding on which the light of further investigation must be thrown. The present short communication attempts to dispose of one of these.

It is well known that in *Amphioxus* the cavities of the typical somites give rise, on the one hand, to all the myocœls except the first pair, and, on the other hand—by the fusion of their ventral moieties on each side—to longitudinal spaces which are the splanchnocœls. But the fate of the so-called “collar cavities” has not hitherto been satisfactorily established. It was known that from the walls of their dorsal parts were formed the first pair of myotomes; but there has been difference of opinion about the behaviour of their ventral parts.

MacBride (1) at first maintained that “the metapleural ‘lymph canals’ found in the atrial folds are the persistent

ventro-lateral extensions of the collar pouches"—having observed this relation to hold in the case of the right collar cavity, and inferring that it was true of the left side also.

Lankester and Willey (4) have described a pseudocœlic origin of the metapleural spaces, and MacBride, in answer to criticism by Lankester and others, returned to this subject in his paper of 1900 (2). Some of the conclusions arrived at, as a result of this re-investigation, were as follows:

"The ectoderm on the external side of these ridges" (i. e. the atrial ridges) "becomes thickened, the cells composing the thickening become clear and glassy and eventually are hollowed out to form a 'lymph canal.' My former statement as to the cœlomic nature of this lymph canal is therefore incorrect.

"The extensions of the collar cavities into the atrial ridges become first separated off as the metapleural cœlom on each side; later this cœlomic space becomes converted into a solid mass of cells from which arise muscular fibres in the neighbourhood of the gill openings, and almost certainly, later, the sub-atrial muscle."

In the following year van Wijhe (5) affirms: "Nach den Verhältnissen beim ausgebildeten Thiere halte ich die Angabe von MacBride aus dem Jahre 1898, nach welcher die Seitenflossenhöhlen in Continuität mit den 'collar pouches' entstehen würden, für richtiger als die spätere Behauptung des selbständigen Auftretens der Seitencanäle."

Finally, MacBride, in his latest contribution to this subject, reiterates his former assertion—made in his second paper—that the collar cavities form spaces in the atrial ridges, longitudinally co-extensive with the pharynx, and distinct from the splanchnocoel (3).

The present investigation was begun at the suggestion of Prof. MacBride, and was carried out in his laboratory. We wish here to make grateful acknowledgment of our indebtedness to him for the assistance he has given throughout the progress of the work, and for the generous permission to make use of his preparations for purposes of comparison.

## MATERIAL AND METHOD.

Four larval stages were examined. Larvæ which had been fixed in Hermann's fluid were obtained from the Naples Zoological Station. They were cut into series of transverse sections by the method of double embedding in celloidin and wax, and the sections,  $4\mu$  or  $5\mu$  thick, were stained variously with Delafield's hæmatoxylin, thionin, alcoholic hæmatein; or with an aqueous solution of picro-nigrosin for the special purpose of making plain the relations of the myosepta.

It is, as other workers have found, difficult to obtain perfectly preserved material; larvæ of the same batch apparently vary in their reaction to the fixative. To obtain reliable results it was found necessary to section a large number of animals, discarding those which showed distension or contraction, and basing conclusions on those alone in which the histological detail was convincing.

Our drawings are, to the best of our ability, faithful reproductions of the appearance of the sections, except that in some of them the irrelevant cytological detail is omitted. They were all made at the level of the microscope stage with the aid of a camera lucida, the magnification being, in each case, that obtained with a 2 mm. apochromatic oil-immersion objective and No. 6 compensating ocular of Leitz.

## DESCRIPTION.

The earliest stage examined was one in which the larvæ show as yet no indication of the formation of a mouth. The left head cavity is a vesicle unconnected with the ectoderm—though in contact with it—the club-shaped gland opens widely into the floor of the enteron, and the endostyle appears as a slightly thickened area of the right side of the swollen pharyngeal region. The formation of somites from the archenteron is still occurring at the extreme posterior end of the animal, and the tail has not begun to grow.

In such a larva the cavities of the collar somites have no

ventral extension round the sides of the fore-gut, and, indeed, in sections in front of the club-shaped gland the mesoblast does not appear at all between the ectoderm and the gut-wall (Pl. 18, fig. 1), save for an occasional isolated cell. In sections a little further back, in the region where the mouth will be formed, the collar somite on either side sends a ventral horn downwards round the gut as a thin plate of cells; but since there are no cavities in these extensions and the myosepta have not yet assumed their characteristic appearance, it is impossible to make out the relations of the somites in this stage.

The right collar cavity is completely separated from the gut, but on the left side there is a virtual communication, marked by the peculiar orientation of the cells of the gut-wall.

Our second (and critical) stage is one in which the mouth has just become established, but is still a mere pore. Pl. 18, fig. 2, shows the appearance of a section about  $5\mu$  behind the blind anterior end of the gut. The collar somites have considerable cavities which extend ventrally on either side of the pharynx. We will first deal with that of the right side. Into its dorsal part projects the mass of the first myotome, the cells of which are already differentiated as muscle; its ventral horn can be traced, with diminishing lumen, to the mid-ventral line. The next section of the series (Pl. 18, fig. 3) shows the ventral horn as before; but in the muscle a crescentic septum has appeared, dividing its mass into an inner and an outer portion. This septum is the first myoseptum, and the inner muscle mass (in contact with the notochord) is the anterior end of the second myotome (i.e., first trunk somite). Succeeding sections show the gradual increase in size of the trunk somite at the expense of the collar somite, as evidenced by the outward and downward migration of the septum (Pl. 18, figs. 4 and 5). Two sections further on (Pl. 18, fig. 6) the cavity of the trunk somite is seen to be well established above the dorsal edge of the septum, and four sections beyond this the septum has just

lost its apical attachment to the gut, so that the cavities above and below now communicate (Pl. 18, fig. 7). The remains of the septum have disappeared in the next section.

It will be plain from this description that the collar cavity of the right side is continuous with the splanchnocœl. It has a more extensive (longitudinally) communication with that space than have the succeeding myocœls, but essentially its relations are the same. We have not found anything comparable to the septum described by MacBride as separating a postero-ventral extension of the collar cavity from the splanchnocœl.

Turning now to the left side of the larva, we see that the first myoseptum is well in advance of that of the right side. In Pl. 18, fig. 2, the ventral horn of the collar cavity is already pushed down to the level of the middle of the notochord, and in tracing sections back the first trunk myocœl is found to be well established dorsally when the section passing through the mouth is reached—the septum between it and the collar myocœl having passed obliquely downwards and backwards to meet the upper lip. The cavity of the upper lip (virtual at this stage) is that of the first trunk somite.

What happens in the lower lip is more difficult to make out. A thickened layer of ectoderm, applied to the left head cavity and to the antero-ventral wall of the gut, suppresses altogether the ventral extension of the somite (Pl. 18, fig. 2); just behind this thickening the somite passes down, its lumen often occluded, to become continuous with the mesoblast of the lower lip, which, in its turn, is continuous with the splanchnocœl (Pl. 18, figs. 3, 4, 5). Again, no septum is observable between collar cavity and splanchnocœl; but, the spaces being for the greater part merely virtual, it is impossible to state with certainty whether there is actual continuity.

The great dilatation of the collar cavities, which MacBride took to be the first appearance of the atrial folds, is, in our opinion, largely a fixation effect. It depends, no doubt, as he

suggests, upon the amount of fluid contained in the cavities, but the actual ectodermal profile we take to be an artifact. On the left side, where the ectoderm is anchored, so to speak—by its fusion with the gut, to form the mouth, and with the head cavity to form the præ-oral pit—the swelling occurs to a lesser degree than on the right side, where no such attachments exist; and in the trunk region, where the cavities of the myotomes are small or virtual, and the close apposition of the walls of the splanchnocoel would preclude osmotic disruption, it occurs not at all. The artificial nature of the distension in question is frequently made apparent by a separation of the extremely thin outer wall of the somite from the ectoderm. This is seen even in so young a larva as that shown in Pl. 18, fig. 1 (q. v.); in later larvæ the action of the fixative is often to rupture the ectoderm on the right side.

The communication between the left collar cavity and the gut, described by MacBride, is indicated in larvæ of this stage (Pl. 18, figs. 2 and 3). It still appears in the majority as a funnel-shaped depression in the dorso-lateral wall of the gut, with sometimes a plug-like fascicle of columnar cells filling it (Pl. 18, fig. 2). It is never, in our sections, a very definite structure, but it gives a characteristic shape to the lumen of the gut, which is generally found to persist through several sections. In the preparations we have examined of a slightly later stage (mouth and one gill established) we have failed to demonstrate its presence; but the swollen, vacuolated condition of the gut cells in this later stage makes it easily possible that the connection, though not recognisable, is present.

In later larvæ still (seven or eight primary gills present) the nephridium of Hatschek appears, as described by Goodrich (6), lying in a space which is apparently a backward prolongation of the left collar myocoel. But whether this space is the original portal of communication, drawn out into a tube by the growth of the larva, and, if so, how the nephridium (an organ presumably of ectodermal origin) comes to lie naked within it, are questions that our materials

do not enable us to answer. Nevertheless, attention should perhaps be called to a constant but inconspicuous feature of our second stage larvæ—the mass of cells marked with a point of interrogation in Pl. 18, fig. 4. They occur just behind the communication above mentioned, and it is tempting to regard them as a proliferation of ectoderm into the first myoseptum and as the rudiment of the nephridium. We cannot, however, assert that this is so.

In conclusion, our acquaintance, limited as it is, with the early stages of development of *Amphioxus* has convinced us of the need of careful experiment with the object of discovering better methods of fixation of the larvæ. This can only be done by rearing them in large numbers in the laboratory—an undertaking never yet achieved. Only so can material be obtained the study of which will give satisfactory answers to the many morphological questions which still remain doubtful.

#### NOTE BY PROF. E. W. MACBRIDE.

The investigation carried out by Messrs. Smith and Newth in my laboratory on the development of body cavities in the larva of *Amphioxus* has led them to results which in some respects are different from those which I have published on the same subject.

In particular they find that the space into which the right collar cavity opens as it sweeps downwards towards the mid-ventral line is the splanchnocœl, and not, as I supposed, a distinct cavity lying external to the splanchnocœl, which later became the cavity of the atrial fold, or, as van Wijhe terms it, the pterygocœl.

After a careful examination of the preparations made by Messrs. Smith and Newth, which were based on better preserved material than was available to me, I have come to the conclusion that these authors are right, and I am prepared to accept their view. A re-examination of my own preparations leads me to believe that the septum which I believed to

divide the splanchnocœl from another cavity external to it is the parietal wall of the cœlom, which in the process of preservation has become separated from the ectoderm.

The facts elucidated by Messrs. Smith and Newth enable us to compare the collar cavity of *Amphioxus* directly with the mandibular cavity of the embryos of *Petromyzon* and the *Elasmobranch* embryo. This cavity has been observed by Hatta to originate in *Petromyzon* from the wall of the gut independently of the outgrowth which gives rise to the myotomes behind, but in both *Petromyzon* and the *Elasmobranch* embryo the mandibular cavity becomes subsequently connected by a long tongue-like ventral extension with the splanchnocœl.

#### LIST OF WORKS REFERRED TO IN THIS PAPER.

1. MacBride, E. W.—“The Early Development of *Amphioxus*,” ‘*Quart. Journ. Micr. Sci.*,’ vol. xl, 1898.
2. ——— “Further Remarks on the Development of *Amphioxus*,” ‘*Quart. Journ. Micr. Sci.*,’ vol. xliii, 1900.
3. ——— “The Formation of the Layers in *Amphioxus* and its Bearing on the Interpretation of the Early Ontogenetic Processes in Other Vertebrates,” ‘*Quart. Journ. Micr. Sci.*,’ vol. liv, 1909.
4. Lankester, E. Ray, and Willey, A.—“The Development of the Atrial Chamber of *Amphioxus*,” ‘*Quart. Journ. Micr. Sci.*,’ vol. xxxi, 1890.
5. Wijhe, J. W. van.—“Beitr. z. Anat. des Kopfregion des *Amphioxus lanceolatus*,” ‘*Petrus Camper*,’ vol. i, 1901.
6. Goodrich, Edwin S.—“On the Structure of the Excretory Organs of *Amphioxus*,” ‘*Quart. Journ. Micr. Sci.*,’ vol. liv, 1909.

#### EXPLANATION OF PLATE 18,

Illustrating Messrs. K. M. Smith and H. G. Newth’s paper,  
“A Note concerning the Collar Cavities of the Larval  
*Amphioxus*.”

#### ABBREVIATIONS.

The asterisk marks the communication between the left collar somite and the gut.

*ch.* Notochord. *c. g.* Club-shaped gland. *esty.* Endostyle. *l. c. s.* Left collar somite. *l. h. c.* Left head cavity. *l. m. 2.* Second myotome of the left side. *l. mc. 2.* Second myocœl of the left side. *m.* Mouth. *n. c.* Nerve cord. *r. c. s.* Right collar somite. *r. h. c.* Right head cavity. *r. m. 1.* First myotome of the right side. *r. m. 2.* Second myotome of the right side. *r. mc. 2.* Second myocœl of the right side. *s.* Septum. *spl.* Splanchnocœl. *t. mc.* Trunk myocœl.

Fig. 1.—Transverse section through the pharyngeal region of a very young larva, showing the relations of the collar somites to the gut. The section is  $4\mu$  thick and was stained with thionin. Note the separation of the outer wall of the right collar somite from the ectoderm.

Figs. 2-7.—Transverse sections through the pharynx of a larva in which the mouth is just formed. Figs. 2-5 are of consecutive sections; between 5 and 6 one section is missed, between 6 and 7 three sections are missed. The preparation from which the drawings were made was overstained in picro-nigrosin to make plain the septa, and this has obscured the cytological detail. For further description see text.

Fig. 8.—Diagrammatical representation of the relations of the cœlomic spaces of the left side of a larva in which the mouth is just formed. The dotted lines show the condition on the right side where the wide opening of the collar somite to the splanchnocœl is not interfered with by mouth or head cavity.